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Negligible Synergistic Effect of β_2-Glycoprotein I on the Reactivity of Antioxidized Low-Density Lipoprotein Antibody to Oxidized Low-Density Lipoprotein

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We conducted this study to investigate whether antioxidized low-density lipoprotein (a-oxLDL) is an antibody to cryptic and/or neo-antigen on β_2-glycoprotein I (GPI), which is introduced by binding to anionic phospholipid, similar to that of GPI-dependent anticardiolipin antibody (aCL) employing a-oxLDL ELISA. We found that no significant optical density differences existed among systemic lupus erythematosus patients, including cases with aCL and/or lupus anticoagulant positivity, before and after the addition of GPI. Our results suggest that a-oxLDL is not an antibody to denatured GPI, but rather to oxLDL. © 1996 Wiley-Liss, Inc.

Key words: oxidized low-density lipoprotein, antioxidized low-density lipoprotein antibody, anticardiolipin antibody, lupus anticoagulant, arteriosclerosis

INTRODUCTION

Oxidized low-density lipoprotein (oxLDL), along with glycosylated-LDL and/or malonaldehyde-LDL, a subset of modified LDL, has recently begun to attract the interest of researchers as one of the causative factors underlying arteriosclerosis [1,2].

As one of the possible mechanisms for the induction of arteriosclerosis, direct and/or indirect injuries of endothelial cells by oxLDL, produced from LDL engulfed by monocytes/macrophages and/or endothelial cells, has been proposed [1,2]. It is possible that oxLDL may acquire a new antigenic property through this process to produce antioxLDL antibody (a-oxLDL). Some researchers [3,4] have reported a high frequency of detection of a-oxLDL in patients with arteriosclerosis.

Two subtypes of anticardiolipin antibody (aCL), a representative antiphospholipid antibody, are known to exist [5]. One is β_2-glycoprotein I (GPI)-dependent aCL, which is thrombogenic, and the other is GPI-independent aCL, which has no pathogenic role [5,6]. Furthermore, it was recently suggested that aCL is not an antibody to phospholipid itself, but is an antibody to cryptic and/or neo-antigen on GPI, which is introduced by binding to anionic phospholipid [6,7].

Vaarala et al. [8] found a-oxLDL in 80% of patients with systemic lupus erythematosus (SLE), including those with aCL. These investigators also noted a correlation between the antibody titers of a-oxLDL and aCL and further confirmed that the majority of aCL was absorbed by oxLDL-lyposomes. From these results, they concluded that aCL and a-oxLDL were antibodies that cross-react with each other. Their findings suggest the possibility that a-oxLDL is an antibody to GPI, which obtains new antigenicity by binding to oxLDL. We conducted the present study to test this hypothesis.

MATERIALS AND METHODS

Subjects

The subjects were 43 SLE patients who met the American College of Rheumatism Association (ARA) criteria for the diagnosis of SLE. Twenty healthy volunteers served as controls.

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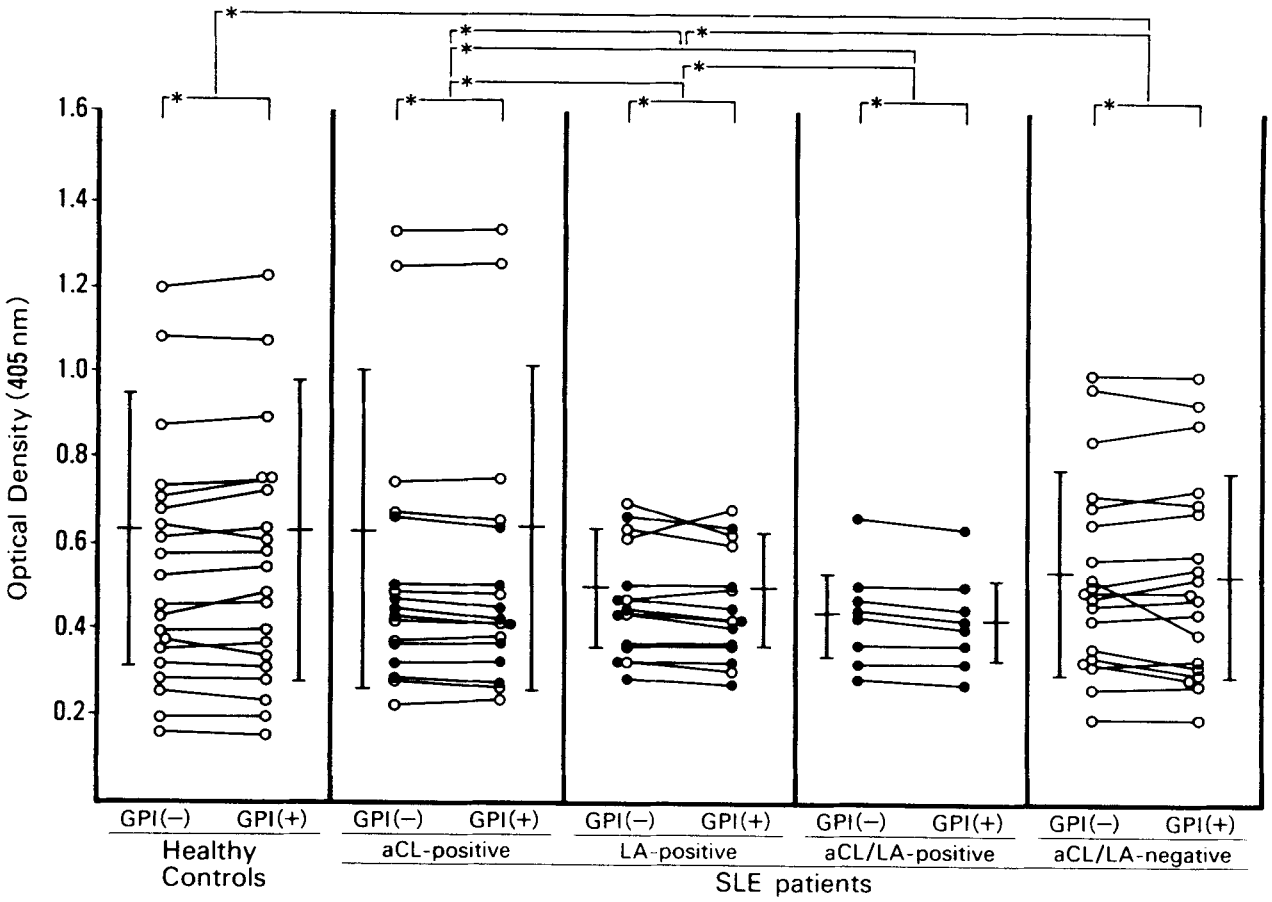

Fig. 1. Influence of β_2-glycoprotein I on the reactivity of antioxidized low-density lipoprotein antibody to oxidized low-density lipoprotein. GPI (−): without β_2-glycoprotein I, GPI (+): with β_2-glycoprotein I, aCL: anticardiolipin antibody, LA: lupus anticoagulant, • — •: patient with both aCL & LA positive, *: no significant difference tested with Student's *t* test.

Methods

LA was screened and positivity determined by the methods recommended by Exner et al. [9]. aCL was measured by the enzyme-linked immunosorbent assay (ELISA) we previously reported elsewhere [10], and a-oxLDL was measured using a test kit (Biomedica, Vienna, Austria) with or without purified GPI (1 μg/well). The results were expressed by readings of the optical density (OD) and the significance of differences between groups of data was tested with Student's *t*-test.

RESULTS

aCL and lupus anticoagulant (LA) were positive in 17 and 15 patients with SLE, respectively, including 8 patients who were positive for both aCL and LA. There were no significant differences among the mean OD readings (±SD) of a-oxLDL without GPI in aCL-positive (0.63 ± 0.38) (OD range: 0.22–1.33), in LA-positive (0.50 ± 0.14) (OD range: 0.32–0.70), in both aCL/LA-positive (0.43 ± 0.11) (OD range: 0.32–0.66) and negative (0.54 ± 0.23) (OD range: 0.20–1.03) SLE groups and healthy controls (0.63 ± 0.33) (OD range: 0.25–1.19) (Fig. 1). No significant increment in the mean OD readings of a-oxLDL after the addition of GPI among aCL-positive, LA-positive, aCL/LA-positive SLE groups, and healthy controls was observed. Furthermore, there was no patient nor healthy control who showed a significant increase in OD readings after the addition of GPI.

DISCUSSION

It has recently been recognized that oxLDL has a pathogenic role in the development of arteriosclerosis [1,2]. Furthermore, it is possible that an antibody to oxLDL also has the synergistic effect of promoting endothelial injury to induce extensive arteriosclerosis [3,4].

Vaarala et al. [8] have reported a high prevalence of a-oxLDL in SLE patients, especially those with aCL, which can cause thrombosis. Their report suggested that

the risk of developing arteriosclerosis in aCL-positive patients with a-oxLDL could be further enhanced. Accordingly, it may be useful to measure aCL and a-oxLDL simultaneously for the prediction of thromboembolic complications. These workers have also suggested cross-reactivity between a-oxLDL and aCL. Their findings prompted us to clarify whether a-oxLDL is reactive to GPI, the antigenicity of which was modified by binding to oxLDL.

We have found that the OD readings of a-oxLDL without GPI in aCL-positive, LA-positive, and/or aCL/LA-positive SLE groups were not influenced by the addition of GPI. Our results suggest that a-oxLDL is not an antibody to cryptic or neo-antigenic GPI like GPI-dependent aCL [6,7], but is an antibody to oxLDL itself. However, further examination employing larger samples may be needed for confirmation.

In contrast to the finding of Vaarala et al. [8], it is interesting to note that there were some healthy controls whose OD readings exceeded the mean value ± 2SD of aCL and/or the LA-positive SLE patient groups. This result may suggest that a-oxLDL could exist not only in a patient group, such as SLE with aCL/LA, but also in a healthy subject group. Our findings are in agreement with those of Salonen et al. [4], who found a-oxLDL in a healthy group, especially in cases with a high cholesterol level and/or with a smoking habit. Differences in the method employed for the measurement of a-oxLDL and/or the population and/or races of the study group may explain why we obtained different results from those of Vaarala et al., but any explanation remains speculative.

Further study to clarify the clinical significance of a-oxLDL, including the mechanisms by which this antibody is produced, may be needed in the future.

REFERENCES

1. Drake TA, Hannani K, Fei SL, Berliner JA: Minimally oxidized low-density lipoprotein induces tissue factor expression in cultured human endothelial cells. Am J Pathol 138:601, 1991.
2. Witztum JL, Steinberg D: Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785, 1785, 1991.
3. Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathyy S, Carew TE, Butler S, Witztum JL, Steinberg D: Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 84:1086, 1989.
4. Salonen JT, Yla-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssonen K, Palinski W, Witztum: Autoantibody against oxidised LDL and progression of carotid atherosclerosis. Lancet 339: 883, 1992.
5. Triplett PA: Antiphospholipid antibodies and thrombosis. A consequence, coincidence, or cause? Arch Pathol Lab Med 117:78, 1993.
6. Matsuda J, Saitoh N, Gotoh M, Gohchi K, Tsukamoto M: Prevalence of β_2-glycoprotein I antibody in systemic lupus erythematosus patients with β_2-glycoprotein I dependent antiphospholipid antibodies. Ann Rheum Dis 54:73, 1995.
7. Matsuura E, Igarashi Y, Yasuda T, Triplett D, Koike T: Anticardiolipin antibodies recognize β_2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. J Exp Med 179:457, 1994.
8. Vaarala O, Alfthan G, Jauhiainen M, Leirisalo-Repo M, Aho K, Palosuo T: Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. Lancet 341:923, 1993.
9. Exner T, Triplett DA, Taberner D, Machin SJ: Guidelines for testing and revised criteria for lupus anticoagulants. SSC subcommittee for the standardization of lupus anticoagulants. Thromb Haemost 65: 320, 1991.
10. Matsuda J, Saitoh N, Gohchi K, Gotoh M, Tsukamoto, M: Distinguishing β_2-glycoprotein I dependent (systemic lupus erythematosus type) anticardiolipin antibody and independent (syphilis type) anti-cardiolipin antibody with Tween 20. Br J Haematol 85:799, 1993.